**REMARKS**

The specification has been amended to include the sequence identification numbers on page 20, lines 20 and 22 as required by 37 CFR § 1.821 and MPEP 2421.02. Additionally a sequence listing in attached hereto.

A declaration under 37 CFR §1.132 by Prof. Baici is included herewith. Prof. Baici's declaration shows the kinetic of production of the catalytically active form of urokinase (*tc-uPA*) as a consequence of long term cell culture incubation (0-96 hours) with butyrate.

This kinetic allows for the recovery of more than 95% catalytically active tc-uPA during a period of about 4 days (96 hours). The 95% catalytically active tc-uPA value was present in the Application as originally filed (see page 9, lines 1-4 and 9-14) and is now claimed to overcome the novelty and nonobviousness issues raised by the Examiner.

Moreover a scientifically sound basis for a new mechanism of action triggered by butyrate is also provided.

The claims of this application have been rewritten in response to the Examiner's rejections. The antecedent basis for these Claims, as well as their relation the former Claims is set forth below.

New independent Claim 81 is directed to a process for producing catalytically active *tc-uPA* by the addition of butyrate analogues to a cell line genetically transfected with a cDNA sequence encoding for the urokinase precursor, wherein the catalytically active *tc-uPA* is more than 95% of the total urokinase secreted by the cell into the supernatant.

Antecedent basis for the new claimed process ("production of catalytically active *tc-uPA*") is found throughout the Application, in particular on page 4, lines 3-5, page 5 lines

6-9, 25-26, 31-32 continuing on page 6 line 1-2 and page 9 line 1-4.

Antecedent basis for the 95% value and for the recitation: "of the total urokinase", introduced for clarity reason, are found on p. 9, lines 1-4 and 9-14. This is where antecedent basis is found for the conversion of the precursor forms to tc-uPA having an overall efficiency above 95%. Additionally, Claim 82 corresponds to former Claim 74, Claim 83 corresponds to former Claim 79, Claim 84 corresponds to former Claim 72, Claim 85 corresponds to former Claim 75, Claim 86 corresponds to former Claim 76 and Claim 87 corresponds to former Claim 77.

Claim 88, which is dependent on Claim 81, is directed to a further step of recovery of the (exhausted) cell culture medium. Antecedent basis is found in the specification page 6, line 11-23, particularly step c), page 8, line 23-32 and page 9, line 15-18. The term "supernatant", which can be found in the pages cited above, has been substituted for "cell culture medium" in all the claims for clarity reasons and in Claim 87 in order to link it to the antecedent basis present in Claim 81. The terms cell culture supernatant and exhausted cell culture medium or supernatant are, however, used interchangeably in the specification.

New Claim 89 refers to the tc-uPA concentration in the exhausted cell culture medium which has been recovered after the optimal time of incubation; said concentration corresponds to at least 4000 IU/ml. Basis is found in the specification, p.8, line 30-32 and p.9 line 1.

Claim 90 corresponds to former Claim 80. The reference to step b) has been deleted because it is no longer necessary.

Claim 91 refers to a process for purifying tc-uPA HMW and tc-uPA LMW from the cell culture supernatant obtained

according to a previous step, which consists of chromatography on a cationic exchanger. The term "consisting" as pointed out by the Examiner, pinpoints that purification of the HMW from the LMW tc-uPA is achieved by a single chromatographic step. In new Claim 91 the use of a cationic exchanger for chromatography has now been explicitly claimed. Antecedent basis for Claim 91 can be found in the specification page 9, line 15-21, p 11, line 2-4, and in the third item of former Claim 71. The nature of the ion-exchanger as a "cation"-exchanger in tc-uPA chromatography is well known in the art of recombinant proteins. Additionally, references describing the nature of the ion exchanger were attached to the Amendment dated December 27, 2002 in reply to the second Office Action (*See e.g.* "Protein Purification" by Scopes R.K., Springer Verlag ed., 2nd ed. 1987 (third enclosure to the reply to the second Office Action)). The references clearly stated that once the isoelectric point of a protein is known, the choice of the ion-exchanger, whether cationic or anionic, is mandatory. In the reference "Protein Purification", ready to use tables such as table 5.4 on p. 114, indicate that an isoelectric point $\geq$ 7 requires a cationic ion exchanger. Since the isoelectric point (pI) of urokinase has been known since at least 1981 (see Miwa et al. (Chem. Pharm. Bull. 29:463-471, 1981; hereinafter "Miwa")) and is comprised in the range from 7.5 to 9.7 g (see also the 2000-2001 Calbiochem Catalogue) the skilled person faced with the problem of separating by ion chromatography tc-uPA (either the HMW or the LMW form since the isoelectric points are very close) would without any doubt use a cation-exchanger. The attached references were published well before the filing date of the present application and therefore are implicitly contained in the Application as filed.

Claim 92 refers to the differential elution of LMW tc-uPA and HMW tc-uPA by the addition of a monovalent cation. The nature of the ion has been explained above in relation to Claim 91, and as being obvious in view of the cationic exchanger of Claim 90. Antecedent basis can be found in former Claim 71 and in the specification page 9, lines 23-32.

In paragraphs 3-5 of the Office Action the Examiner objected to Claims 69 and 73 as being grammatically incorrect.

Reconsideration is requested.

Claims 69 and 73 have been cancelled by this amendment and it is therefore believed that the objections are moot. The new claims that have been added by this amendment do not include the grammatical mistakes of the previous claims.

In paragraph 6 of the Office Action, the Examiner rejected Claims 72, 75-78 and 80 under 35 USC § 112, second paragraph.

Reconsideration is requested.

Claims 72, 75-78 and 80 have been cancelled by this amendment and it is therefore believed that this rejection is rendered moot. In new Claim 84, corresponding to former Claim 72, the term "comprised" in the recitation "comprised from 0.1 to 20 mM" has been removed. The rejection of Claims 75 and 76 due to lack of antecedent basis has been clarified in new Claims 85 and 86 (corresponding to former Claims 75 and 76). The temperature is defined in Claim 85, and Claim 86 is dependent on Claim 85. The rejections based on the reference to "step a and step b" are no longer applicable because all references to step a and step b have been removed from the new claims.

In paragraph 8 of the Office Action the Examiner rejected Claims 69-80 under 35 USC §112, first paragraph.

Reconsideration is requested.

Claims 69-80 have been cancelled by this amendment and it is therefore believed that this rejection is rendered moot. However, Applicant will still address these issues in regards to new Claims 81-92.

The issues raised in paragraph 8 of the present Office Action are rendered moot specifically with reference to the issue of lack of enablement by the explicit recitations as set forth in the new set of claims. In particular, the protein precursor has been amended to specify the urokinase precursor, and the "ionic-exchange" chromatography has been amended to a "cationic-exchange chromatography".

It is believed that these revisions of the previous claims will place the present application in condition for allowance.

In paragraph 9 of the Office Action the Examiner rejected Claims 69 and 70 under 35 USC §102(b) as being anticipated by Okabayashi et al. (Cell Struct. Funct. 14:579-586, 1989; hereinafter "Okabayashi")

Reconsideration is requested.

Claims 69 and 70 have been cancelled by this amendment and it is therefore believed that this rejection is rendered moot. However, Applicant will address these issues with regard to new Claims 81-92.

The scope of protection of new Claim 81 has been amended to recite a "**process for the production of recombinant catalytically active two chain urokinase** (tc-uPA) by addition of alkanoic acids to the culture medium of a cell line which has been genetically transfected with a cDNA sequence... **characterized in that at least 95% of the total urokinase is catalytically active tc-uPA.**"

The process now addressed in Claim 81 is new with respect to Okabayashi, which discloses a process for increasing the

overall production of urokinase by adding butyrate to a cell culture carrying the urokinase cDNA.

Claim 81 is also novel with respect to the inherent characteristic of the urokinase produced in the Okabayashi paper. In Okabayashi, the conditioned CHO supernatant is recovered only 24 hours after the addition of butyrate (*see* Okabayashi page 581: "At a given time (usually 24 hours), the incubated media (conditioned media) was taken for the assay"). As shown in figure 2 of the enclosed Declaration, 24 hours after the addition of butyrate the conversion of scuPA to tc-uPA is only about 67%. Therefore this value at maximum, and at least in the hands of the Applicant, is the percentage of catalytically active tc-uPA which Okabayashi may have obtained, even though not explicitly disclosed. In this regard the applicant believes that Okabayashi does not disclose any value of catalytically active urokinase as far as no value at all could be directly or unequivocally derived from his paper.

In any case the 67% value is far from the one presently claimed in Claim 81, said value in Claim 81 being greater than 95%. This conversion value is reached after a butyrate incubation time of 72 to 96 hours on the cells, a time range which is comprised in the preferred time of incubation (48-200 hours) disclosed on p. 6, lines 19-20, and p.8 lines 30-32 of the Application and presently in Claim 87.

However, in presently filed Claim 81, the reference to butyrate incubation time has been deleted and replaced by the value of 95% catalytically active tc-uPA.

It is the Applicant's belief that Okabayashi does not anticipate Claim 81, and therefore Claim 81 is in proper form for allowance.

However it is also in the Applicant's opinion that the amendment of the process to a preferred time of incubation of butyrate in the cell culture (48 to 200 hours), could have contributed substantially to the novelty of former Claim 71 with respect to the Okabayashi explicit disclosure (24 hours maximum).

In view of the above comments, reconsideration of the novelty rejection of independent Claim 81, and the claims dependent thereon is respectfully requested.

In paragraph 10 of the Office Action the Examiner rejected Claims 71-76 and 80 under 35 USC §103(a) as being unpatentable over Okabayashi in view of Nobuhara et al. (J. Biochem. 90:225-232, 1981, hereinafter "Nobuharar") and Miwa et al. (Chem. Pharm. Bull. 29:463-471, 1981; hereinafter "Miwa"). In paragraph 11 the Examiner rejected Claims 77-79 under 35 USC §103(a) as being unpatentable over Okabayashi in view of Nobuhara and Miwa as applied to Claims 71-76 and 80 above, and further in view of Hu et al. (Sheng Wu Gong Cheng Xue Bao 16:387-391, hereinafter "Hu".

Reconsideration is requested.

Claims 71-80 have been cancelled by this amendment and it is therefore believed that this rejection is rendered moot. However, Applicant will address these issues with regard to new Claims 81-92.

The amendment of the claims to point out a process yielding 95% active tc-uPA has been introduced in Claim 81. The Applicant believes that this renders moot the rejections in paragraphs 10 and 11.

In addition, the Applicant has shown (see page 3 of the attached Declaration) that an unexpectedly efficient conversion to the mature form of a precursor protein, correlates with a surprising effect triggered by butyrate:

i.e. activation of the Matrix-Metallo Proteases (MMP) class of enzymes.

This effect, most probably representing the mechanism underlying the present invention, allows the final technical effect of more than 95% urokinase precursor conversion to the catalytically active tc-uPA.

Neither the final effect or the underlying mechanism could be predicted from the prior art which discloses only an overall increase in urokinase production, see Okabayashi either alone or in combination with Hu, wherein catalytically active tc-uPA is disclosed to represent only 10% of the total urokinase recombinantly produced. Therefore according to the previous references obtaining such a high percent of catalytically active *tc-uPA* was a totally unexpected result.

With regard to the obviousness issue related to former Claims 71 and 80 (now respectively Claims 91 and 92), the Applicant wishes to point out that in the new claims above, Claim 91 is related to a purification process for separating HMW form LMW *tc-uPA* consisting in a cationic chromatography performed on the (exhausted) cell culture medium of butyrate treated cells to indicate that before ionic-chromatography the starting material is not formerly gel-filtered on Sephadex G100 and on G75.

The possibility of achieving HMW and LMW tc-uPA separation directly from the cell culture medium of a recombinant eukaryotic cell line, even by cationic chromatography known in the art, without, for example, a step of prior concentration of the cell culture medium, represents a completely unexpected result. As a matter of fact, concentration of secreted recombinant proteins in the supernatant are usually relatively low (usually below the

order of magnitude of gram/Liter) and this represents a very well known problem for purification purposes.

As noted in the amendment dated December 27, 2002, the concentration of urokinase in the starting material is very different in the present Application than the concentration of urokinase in Miwa. Miwa's starting material is a partially purified urinary extract (see p. 463 first line of Materials) wherein the urokinase concentration is about 3000 fold higher (see p. 464, Results and Discussion, first paragraph, l.1-2) than in the CHO supernatant of the present Application (about 0.05-0.1 mg/ml, corresponding to about 4000 IU/ml).

Therefore, in view of Miwa's teaching, the skilled artisan would consider the achievement of a good separation between HMW and LMW tc-uPA from a supernatant obtained by fermentation of a recombinant cell line by a simple chromotographic step to be unobvious and unexpected. This clearly points to the patentability of Claims 91-92.

The starting material is critical in setting out the purification condition. In new Claim 91 the starting material is mentioned and it is the cell culture supernatant obtained as described in independent Claim 81. The concentration of tc-uPA in this medium has also been indicated in new Claim 89 by reference to the International Units measured by a chromogenic assay as described in the specification, p. 17, lines 17-18.

Based on the Declaration, new claims and above remarks, applicant respectfully submits that new Claims 81-92 are allowable over the prior art and that the present application is in proper form for allowance. Reconsideration of these rejections is requested in view of this amendment.

For these reasons, it is requested that the grounds for rejection be withdrawn.

An early and favorable action is earnestly solicited.

Respectfully submitted,

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MARKED UP COPY OF AMENDMENT TO SPECIFICATION

Kindly rewrite the paragraph that begins on page 20, line 17 as follows:

The correct processing at the N-terminal was confirmed through the Edman degradation of purified HMW tc-uPA. As expected, the $NH_2$-terminal sequence of the B-chain was determined as:

**- seq IDN3 IIGGEF-,**

whereas the $NH_2$-terminal sequence of the A-chain was, as expected:

**- seq IDN4 SNELHQ-,**

These data demonstrated that the proteolytic cleavage occurs exactly and specifically at the $Lys^{158}$-$Ile^{159}$ bond, and $Lys^{158}$ is correctly removed from the rest of the molecule. Moreover, the analysis of the peptide mapping confirmed the existence of correct $NH_2$- and C-termini of both A- and B-chains of the recombinant tc-uPA HMW.